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APPENDIX U

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by

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RADIOBIOLOGICAL EXPERIMENTS WITH PLANT SEEDS ABOARD THE BIOSATELLITE KOSMOS 1887

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Abstract—The effects of spaceflight factors on the seeds of *Arabidopsis thaliana* and *Crepis capillaris* were studied provided with various protective measures: the seeds were located inside the satellite and in open space, protected with aluminium foil and also exposed without the foil cover. When the seeds were in open space without any protection, their viability was found to be suppressed; the survival rate and fertility of plants grown from these seeds were also diminished. An increase in the frequency of chromosome aberrations (CA) and in the number of multiple injuries was registered in this case. Experiments with the aluminium foil shielding showed a decrease in the suppression of the seeds' viability, but mutational changes were found to be even more increased, while the survival rate and fertility of the plants decreased. An increase in the thickness of shielding resulted in a decrease in the effects up to the level of the control, except for the effects connected with CA and fertility of the plants. Analysis of the results shows that these impairments can be ascribed to the action of single heavy charged particles (HCP). The seeds can be thus regarded as an integral biological 'dosimeter' which allows estimation of the total effects of radiation, ecological and biological factors.

INTRODUCTION

EXPERIMENTS conducted aboard spacecraft have shown that biological objects can be severely damaged by HCP. There is strong evidence for the existence of direct correlations between the hitting of the object with the particles and the injuries observed (Grigoriev and Nevzgodina, 1978; Grigoriev, 1982; Nevzgodina *et al.*, 1984; Maximova, 1985). The experiments made in open space make it possible to analyze the total effect of ionizing and UV-radiations of the solar spectrum light and other spaceflight factors. At the present time, in connection with the problem of ozone holes, the studies of those electromagnetic radiations and cosmic rays which fail to reach the surface of the Earth being absorbed by the atmosphere have become increasingly important. Those experiments are to be conducted at altitudes exceeding 100–150 km. They require the employment of spacecraft to expose the studied organisms both to the conditions of spaceflight and to the factors of open space.

EXPERIMENTAL

The experiments conducted aboard the biosatellite Kosmos 1887 constitute an integral part of the programme of radiobiological research implemented by means of biosatellites of the Kosmos series. The task of the experiment was to study the effects of spaceflight on plant seeds provided with various types of protection. The experiments were carried out both inside the satellite and in open space. In the latter case, the seeds were fixed to a removable cover of a container located on the outer surface of the satellite. A monolayer of seeds fixed with PVA to the cellulose-nitrate plates was exposed to open space either without any protection or covered with aluminium foil of thickness 15 μm (1.76 mg cm^{-2}). To estimate the absorbed doses, thermoluminescent dosimeters were used. Throughout the flight a dose absorbed by the sample stored inside the spacecraft amounted to 6.7×10^{-3} Gy. A dose absorbed by the seeds exposed to open space and protected with foil ranged from 15 to 36 Gy, depending on the location of the seeds on the cover of the container. After landing, the seeds were exposed to low temperatures from -15 to -18°C. The air-dried seeds of the model plants of *Arabidopsis thaliana* and *Crepis capillaris* were employed in the experiments. Numerous tests developed for each of these plants have proved to be complementary; they allow evaluation of both the direct effect of the factors under study and future results by means of a number of indices which

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characterize the viability, fertility and mutability of plants. The viability was estimated by the germination energy of the *Crepis* seeds, as well as by the germination ability of both plants and also by the survival rate of the *Arabidopsis* plants. The mutability was estimated by the frequency of aberrant cells and by the multiple rearrangements which occur at first division metaphases in the root meristem cells of *Crepis*, as well as by the death of the seedlings at a cotyledon phase caused by rough chromosome aberrations. The mutability was also estimated by the frequency of recessive mutations of *Arabidopsis* (Ivanov, 1974; Anikeeva *et al.*, 1983). Taking into account the fact that differences in the doses absorbed by the seeds located at various sites of the cover outside the satellite do not exceed those detected by various dosimeters in the case of one and the same distribution, and also that the results obtained in different variants of the experiment do not differ significantly, the authors found it possible to summarize the results irrespectively of the distribution of the seeds over the cover of the container. The indices obtained in the laboratory and transport controls were also summarized, as they failed to show any statistically significant differences.

RESULTS AND DISCUSSION

The results of the action of spaceflight factors on the air-dried plant seeds are shown in Table 1. In the case of unprotected seeds located outside the satellite all the indices under study were found to deteriorate, as compared to the ground-based control, except for the frequency of recessive lethals in *Arabidopsis* (this index was found to be diminished, although these changes were statistically insignificant). This can be attributed to the selection of mutants which results from a decrease in the viability and fertility of the plants grown from the exposed seeds. Noteworthy is the fact that the germination energy of *Crepis* was found to be considerably decreased (by a factor of two) and the germination ability of both plants was found to be slightly lowered (by 10%); a 39-fold decrease was also found in the frequency of CA in *Crepis*, as compared to the ground-based control. Besides this, a great number of cells with multiple rearrangements were seen, which was not the case with the ground-based control. A thin layer of protective foil was shown to decrease the effect of open space on the viability of the seeds. The energy and ability of germination were not different from those of the control. As to all the other indices under study (except for the survival rate of *Arabidopsis*) the authors observed even greater influence of spaceflight factors, as compared to the variant without any protection. Further increase in shielding (e.g. locating the seeds inside the satellite) resulted in elimination or significant depression of these effects. As compared to the ground-based control, a statistically significant increase in the frequencies of CA and multiple rearrangements was registered in *Crepis* only. The number of unfertilized seedbuds in *Arabidopsis* was found to be increased, too.

Table 1. Effects of space on seeds of *Crepis capillaris* and *Arabidopsis thaliana* (%)

Biological characteristics of tests	Investigation tests		Control	Flight		
				Inside the satellite	Outside the satellite: Shielding	Outside the satellite: Without shielding
Viability	Germination energy of *C. capillaris*		88.98 ±	90.26 ± 1.18	91.11 ± 1.24	48.72 ± 2.30
	Germination ability of	*C. capillaris*	97.04 ±	97.29 ± 0.65	97.54 ± 0.677	89.61 ± 1.41
		A. thaliana	98.36 ±	97.63 ± 0.68	95.16 ± 1.03	89.44 ± 1.35
	Survival rate of *A. thaliana*		73.68 ±	69.31 ± 2.05	62.50 ± 2.33	61.04 ± 2.14
Mutability	Frequency of aberrant cells in *C. capillaris*		0.50 ±	1.52 ± 0.27	24.14 ± 0.44	19.55 ± 0.61
	Frequency of cells with multiple aberrations in *C. capillaris*		0.00	0.05 ± 0.05	3.61 ± 0.33	3.03 ± 0.28
	Death of the seedlings *A. thaliana*		17.31 ±	20.20 ± 1.84	27.36 ± 2.19	24.68 ± 1.99
	Frequency of embrional lethals in *A. thaliana*		3.00 ±	2.91 ± 0.14	4.70 ± 0.20	2.77 ± 0.11
Fertility	Unfertilized seedbuds of *A. thaliana*		39.85 ±	46.76 ± 0.32	50.43 ± 0.35	43.95 ± 0.32

A tendency to a decrease in the survival rate was shown in *Arabidopsis*. Which of the factors are responsible for the phenomena observed? In the variant without any protection the seeds were exposed to a complex of factors of open space: UV- and ionizing radiations, visual light, infrared light, vacuum, and microgravitation. As was shown in our experiments (Anikeeva *et al.*, 1983; Vaulina *et al.*, 1984) the last-mentioned factor fails to have any significant effect on the plant seeds if the duration of their exposure to microgravitation is short. Vacuum and low temperatures also failed to show any effect on the viability, mutability, and other indices. However, high temperatures may have an adverse effect on the seeds, and vacuum may enhance the effects of UV-light and ionizing radiation (Imshenetsky, 1975; Vaulina *et al.*, 1974). Unfortunately, it was not feasible to measure the radiation doses absorbed by unprotected seeds exposed to open space, since the luminescent dosimeters used for this purpose would have been ruined under those conditions. A thin foil cover protects the seeds from the UV-light and delays their drying; it creates possible additional irradiation of the seeds by means of δ-electrons formed when both high- and low-energy cosmic rays are passing through the foil (the so-called anomalous component with the maximum of the energy spectrum at 12–16 MeV nucleon^{-1}). Deceleration of the ions of the anomalous component (mainly oxygen) which occurs when they are penetrating the foil and the seed skin results in maximum losses in energy directly in the area of the seed location. In this variant the ionizing radiation dose registered was shown to be the highest. An increase in the thickness of the protective cover (the wall of the satellite) leads to a decrease in the radiation dose absorbed due to the reduction of the general flow of particles and hardening of the spectrum. In this case the radiation dose registered is rather low. And to what do the biological results testify? The whole complex of open space factors has an adverse effect on all the inherent indices. On the one hand, protection against the UV-light eliminates the effects connected with the suppression of the seeds' viability. On the other hand, an increase in the mutability and a decrease in the survival rate and fertility of plants in the experiments conducted in open space (with the foil and without it) are related to the effects of HCP. The impact of HCP is evidenced by the large number of cells with multiple chromosomal injuries. A rise in the temperature and exposure to vacuum could modify the effects observed in these variants. In the variant with the foil these effects were found to be more pronounced. Unfortunately, it was impossible to use dosimeters in open space for measuring the doses absorbed by unprotected seeds; however the biological results obtained make it possible to believe that the radiation doses in this case could be lower, as compared to the variant with the foil protection. The dose registered in the variant inside the satellite was far too small to be responsible for the effects observed. The presence of cells with multiple chromosomal injuries suggests that the impairments revealed are connected with the action of single heavy charged particles. However they are not very numerous in this case, as compared to the variants when the seeds were located inside the satellite. This is evidenced by the reading of the detectors and also by a decrease of one order of magnitude registered in the total frequency of CA, as well as by a very significant (60–70-fold) decrease in the frequencies of multiple aberrations which was lacking in the control. Multiple aberrations appear in the cell when it acquires a large amount of energy. Thus, for instance, when the cells are irradiated with gamma-rays these aberrations appear only when the doses are as high as 30 Gy. It is worth noting that the plant seeds not only exhibited all the changes in all the variants of the experiment, but also responded differently to the action of open space factors (i.e. to UV- and ionizing radiations). None of the existing dosimeters can cover such a broad spectrum of radiations either totally or quantitatively. As to the radiation-dependent effects revealed, those plants can be regarded as an integral biological dosimeter, the application of which necessitates a more detailed study to be carried out, so as to establish the dependence of the 'dosimeter readings' on the effects of the radiation and also on the ecological and biological (age, etc.) characters. The changes found are quite obvious. However, not all of these changes are statistically significant, as compared to the control and to one another. We believe that this can be attributed to the fact that at small absorbed doses the sampling of the studied materials is insufficient. Based on the analysis of the adequacy of the sampling volume, it has been shown that for obtaining statistically significant information on the radiation-induced changes in the cells by means of cytogenetic analysis it is necessary to have such a quantity of the irradiated and then analyzed biological objects which would be no less than a certain minimum volume of sampling, J_{min}. When solving the problem of prognosticating the minimum volume of sampling, J_{min}, by a number of biological objects required for the irradiation and analysis, we took into account the expected radiation dose absorbed, the fluctuations of the energy absorbed in the responsive volumes of cells, as well as the biological specificity and stochastics of the objects under study. Thus, it has been established that

$$J_{min} = \frac{t^2(P)}{\delta^2 \lambda(N,D)} \times \left[\frac{1}{N} \frac{\bar{\sigma}_n^2(D)}{\bar{n}^2(D)} + \left(1 + \frac{1}{N} \frac{\sigma_\varphi^2(D)}{\bar{\varphi}^2(D)} \right) \frac{\sigma_k^2}{\bar{k}^2} \right], \quad (1)$$

where $\bar{n}(D)$ is the expected mean number of aberrations in the cell at a dose of D which can be described by the following equation:

$$\bar{n}(D) = \bar{k}\bar{\varphi}(D). \quad (2)$$

Table 2. The volume of sampling N^* and J^*_{min} at low doses for seeds of *Crepis capillaris* (L.) Wallr. with the relative standard variance $\sigma_k/\bar{k} = 0.2$, irradiated with protons of kinetic energy 9.2 GeV

D, Gy	J^*_{min} ($N = 25$)	N^*
0.5	17.8 ± 2.6	1098 ± 164
1.0	8.9 ± 1.3	549 ± 82
2.5	3.6 ± 0.5	219 ± 33
5.0	1.8 ± 0.3	110 ± 16

The other values in formula (1) are: $t(P)$ is the solution to the equation $\Phi(t) = P$, where $\Phi(t)$ is the integral of probabilities, P is the level of confidence, δ is the pre-set maximum relative error of the center of expected empirical distribution of aberrations in the cell after its irradiation, N is the number of analyzed cells in each biological object; $\bar{k}$ and δ_k^2 are the mean value and the dispersion of the coefficient of transition from the energy absorption to the effect, $\delta_*^2(D)$ is the expected dispersion of the number of aberrations in the cell at a dose of D; $\delta_\varphi^2(D)$ is the expected dispersion of the function φ at this dose; $\lambda(N,D)$ is the probability of the fact that after the radiation exposure to a dose of D the cell of the biological object analyzed will contain not less than N of the observed (metaphase or anaphase) cell. The given probability can be found experimentally. When the doses are low this probability does not actually depend on the quality and level of the doses absorbed. The relative standard variance $\delta_k/\bar{k}$ is determined at a high dose, D, and for a large number of cells, N, when the stochastics of the absorption energy can be neglected in comparison with the biological stochastics of the objects under study. To estimate the relative standard variance $\delta_k/\bar{k}$ the empirical square variance $S_{N(J,N)}(D)$ is used. The latter can be found from the formula given in Cocren (1976) for the independent experiment with model radiation. For this purpose the biological objects are chosen from the same assemblage as for the cytogenetic studies with planned conditions of irradiation. Practical application of formula (1) was considered with respect to the air-dried seeds of *Crepis capillaris* irradiated with protons with kinetic energy of 9.2 GeV at the stage G_0 of the cell cycle. The obtained data are given in Table 2. In spite of the particular character of these data, their analysis makes it possible to suggest that for obtaining statistically significant information on cytogenetic effects in the cells exposed to small doses of irradiation (e.g. inside the satellite Kosmos 1887) a vast volume of sampling is required as to the number of biological objects (seeds). The minimum value of this volume, J_{min}, depends considerably on the level of confidence P, the probability $\lambda(N,D)$, and the value δ. Therefore, it would be preferable to calculate the volume J^*_{min}. A correlation between the volumes of samplings J_{min} and J^*_{min} is as follows:

$$J^*_{min} = J_{min}\lambda(N,D)\delta^2/t^2(P). \quad (3)$$

Correspondingly, the volume of sampling N^* is described by the equation:

$$N^* = \frac{\sigma_*^2(D)}{\bar{\varphi}^2(D)}\left[0.1\,\frac{\bar{\sigma}_*^2(D)}{\sigma_k^2\sigma_\varphi^2(D)} - 1\right]. \quad (4)$$

For $N \leqslant N^*$ the biological stochastics of the objects under study can be neglected. For $N^* < 1$ they should be taken into account for any N. As seen in Table 2, when the doses of irradiation and the biological stochastics are low, the ratios of the volumes of samplings J^*_{min} are inversely proportional to the dose ratios. A similar picture is observed for the objects of the samplings N^* under the indicated conditions of irradiation. Expression (1) allows more detailed planning of cytogenetic studies carried out in the radiation fields of HCP and facilitates the appropriate choice of the biological object itself. Along with this it shows that before setting the general experiment it is necessary to carry out some auxiliary calculations and experimental studies.

SUMMARY

Thus, as compared to the ground-based control, in the case with the seeds located outside the satellite without any shielding, the plants' viability, survival rate, and fertility were found to be considerably decreased. In this case there is also a significant increase in the cytogenic effect. The variant of the experiment when the seeds were located outside the satellite and covered with foil was characterized by even greater effects, as compared to the variant with unprotected seeds located outside the satellite. Comparison of these two variants (with and without the foil protection) shows even greater decrease in the viability and fertility of the plants, as well as an increase in their mutability revealed by the CA test and also by the test of embryonic lethals. The viability of the seeds in this variant corresponds to that in the control. For the seeds located inside the satellite the researchers observed a three-fold increase in the mutability, as compared to the control; this was determined by the test of CA and by the decrease in the fertility of the plants grown from exposed seeds.

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